179

DOES STORED HPC, APHERESIS PRODUCT NEED AN EXPIRATION DATE? GRAFT CELL VIABILITY AFTER LONG TERM STORAGE

Merchant, M.[1], Huang, W.[2], Olszewski, M.[2], Kletzel, M.[1,2] [1] Northwestern Memorial Hospital & Feinberg School of Medicine, Chicago, IL; [2] Children's Memorial Hospital & Robert H Lurie Comprehensive Cancer Center, Chicago, IL

Background: Regulatory & accrediting organizations require that stored HPC products be labeled with an expiration date. Stem Cell Transplant Program at Children's Memorial Hospital has been collecting HPC, Apheresis products since 1992 with signed donor consent for storage and discard. Though products were labeled with a 5 year expiration date, cells were not discarded until 2006, for want of storage space. We identified 54 products which could be discarded as their intended recipients had died. Products were in liquid nitrogen storage for a period of upto 15.2 yrs. Cells were thawed & viability testing done, as per written procedure.
Objective: We undertook this opportunity to discard products to document effect of long term storage on graft cell viability.
Method: Donor products were collected by HPC, Apheresis between 1992 & 2003. Pre-freezing viability done using Trypan Blue Dye Exclusion test was between 96-100% (median 97.3%). Products were mixed with equal quantity of cryoprotectant [3 parts TC-199 without phenol red and 2 parts each of 10% Dimethylsulfoxide (DMSO) and Autologous serum]. Heparin was used as anti-coagulant. Cells were frozen using a controlled rate freezing program and stored in liquid nitrogen. Product bags were thawed in a water bath between 37 - 40°C, gently kneading the contents. Post thaw samples were re-tested for viability, using Trypan Blue. Statistical evaluations were done on SigmaStat 3.5.
Result:
Table 1.

Years in Storage	# of Products	Viability Range	Viability Median	P-vlaue	Pearson's Correlation (R)
Total (0.1- 15.2)	54	68- 99	90.29%	0.484	-0.0972
0.1- 5.0	27	77- 97	91.03%	0.478	-0.142
5.1- 10.0	12	68- 98	88.58%	0.526	-0.204
10.1- 15.2	15	79- 99	90.33%	0.723	-0.0999

Post thaw median viability was 90.3% (range68- 99%). Of 54 products, 37 products (69%) had viability > 90%; 51 (94%) had viability > 80%; only 3 (6%) had viability below 79%.

15 products (28%) were in storage for < 3.0 yrs; 12 products (22%) for 3.1 to 5.0 yrs; 12 products (22%) for 5.1 to 10.0 years; & 15 products (28%) were stored for > 10.1 yrs. Statistical evaluations revealed no significant relationship between storage time & cell viability.
Discussion: Total nucleated cell viability was measured as an indicator of graft composition. 94% of products retained a viability of > 80% on long term storage. Difference in pre freezing & post thaw median vibility (7%) can be attributed to the freezing process itself. Since HPC, A grafts are capable of engraftment regardless of storage time or percentage of viable cells, stored HPC, A products may actually have a long expiration period.
Conclusion: Long term storage of HPC, A products in liquid nitrogen does not result in any significant cell loss and may not need an expiration date.

180

IMMUNOMAGNETIC SELECTION OF CD8+ MEMORY CELLS FOR THERAPEUTIC APPLICATIONS

Armstrong, R.[1], Lowsky, R.[2], Strober, S.[2], Sheehan, K.[1] [1] Stanford Hospital & Clinics, Stanford, CA; [2] Stanford University, Stanford, CA

Infusion of allogeneic T cells, administered as donor leukocyte infusions (DLI), has widespread acceptance as immunotherapy for relapsed disease after BMT and as a means of increasing donor chimerism in allogeneic recipients experiencing graft resistance. However, recipients of DLI are at risk of GVHD primarily due to the presence of alloreactive CD45RA+ naive T cells in the infused cells and DLI is not an option for patients who develop GVHD post-transplant. Mouse models indicate that GVHD results primarily from naive donor T cells whereas T cells expressing memory phenotypes appear to promote donor chimerism without GVHD. To assess the feasibility of isolating CD8+ memory cells from apheresis collections as an alternative to standard DLI, a tandem immunomagnetic selection strategy was used with CD45RA depletion followed by CD8 enrichment. The resulting cells were predominantly CD8+/CD45RO+/CD44+/CD49d+/CD62L- indicating an effector memory (T_{EM}) subset. Cytokine secretion by *in vitro* stimulation with third party cells showed the selected cells produced high levels of IFNγ and TNFα but little detectable IL-2, IL-4, or IL10 consistent with the effector memory classification. Post-selection recovery was typically > $200x10^6$ cells providing doses of CD8+ memory cells comparable to their representation in high dose DLI. A clinical trial to assess the effectiveness of these cells to improve donor chimerism in patients showing graft resistance is planned.

181

MOBILIZATION OF CD34+ CELLS WITH GRANULOCYTE COLONY STIMULATING FACTOR (GCSF) VERSUS GCSF PLUS PLERIXAFOR IN PATIENTS WITH PRIMARY IMMUNODEFICIENCY DISORDERS (PIDS) UNDERGOING AUTOLOGOUS PERIPHERAL BLOOD STEM CELL (PBSC) COLLECTION

Kang, E.M.[1], Yau, Y.Y.[2], Maxwell, S.[1], DeRavin, S.S.[1], Borge, P.D.[2], Malech, H.L.[1], Leitman, S.F.[2] [1] National Institutes of Health, Bethesda, MD; [2] National Institutes of Health, Bethesda, MD

Peripheral blood CD34+ hematopoietic stem cell (HSC) mobilization with GCSF is the established method for PBSC collection. In 2009 the FDA approved Plerixafor, a CXCR4 antagonist, to enhance mobilization in patients with myeloma and lymphoma. Many patients with PIDs mobilize poorly, limiting autologous PBSC-based treatments. We therefore added plerixafor to our standard mobilization regimen of GCSF, 10 mcg/kg daily for 5 days. Peripheral CD34 counts were assessed on days 4 and 5, and a day 4 CD34 count < 20 cells/uL was used as criteria for addition of plerixafor 240 mcg/kg 11 hours prior to apheresis. Targeted CD34+ yield was 4×10^6 cells/kg. From 1998 to 2010, 28 PID patients, underwent 48 mobilization cycles (1-6 cycles/patient) during which 83 collections were performed (1-3 aphereses/cycle). 16 of 28 patients received GCSF only during 36 mobilization cycles, undergoing 68 procedures (1.9 procedures/cycle, range 1-3). Mean (±SD) circulating CD34 count on day 5 was 31±25 cells/uL (range 2-141) and day 5 product content was 223±208 × 10^6 CD34+ cells, for a cell dose of 4.7±4.3 × 10^6 CD34/kg (range 0.3-18). During 16.4±4.7 liters processed (LP), mean procedure yield was 13.5±10 × 10^6 CD34+ cells/LP. 3 patients had such poor mobilization that further apheresis was considered futile. Since adding plerixafor, 9 of 13 mobilizations met criteria for its use. In these, the day 5 CD34 count was 57±34 cells/uL (range 15-98) and the day 5 product had 342±295 × 10^6 CD34+ cells (range 55-842), for a cell dose of 9.9±8.2 × 10^6/kg (range 2.2-24). During 15.7±7.1 LP, mean yield was 20.9±14.0 × 10^6 CD34+ cells/LP. 3 patients underwent initial mobilization with GCSF alone, then with plerixafor on a subsequent cycle. In one patient, day 5 CD34 counts were 11 vs 25/uL, respectively, and apheresis yields were 110 vs 231 x10^6 CD34s (5 vs 11.6 x10^6 CD34+ cells/LP, and 1.6 vs 3.1 × 10^6 CD34+ cells/kg). In patient two, the day 5 CD34s were 2 vs 15/uL, and product contents were 9 vs 55 × 10^6 CD34s (0.4 vs 2.2 × 10^6CD34/kg). The third patient had undergone multiple GCSF mobilizations, resulting in a mean day 5 CD34 of 41/uL, vs 88/uL with plerixafor. Product content was twice as great with the combination versus GCSF alone. The addition of plerixafor to GCSF more than doubles the peak CD34 mobilization response and apheresis yield of autologous PBSC collections in patients with PIDs and should greatly facilitate PBSC-based treatment strategies.

182

LONGER INCUBATION TIME OF FROZEN CORD BLOOD CFU-GM CULTURES ALLOWS CELLS TO RECOVER AND SHOW SIMILAR GROWTH COMPARED TO CFU-GM ASSAYS PERFORMED ON FRESH CELLS

Stockinger, S., Flannery, S., Miller, C.N., Miller, S.N., Freed, B.M. University of Colorado, Aurora, CO

Assessment of cryopreserved umbilical cord blood potency is an important criterion for hematopoietic stem cell engraftment.